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Available data indicate that the genetic susceptibility for low bone mass is present very early in life. The aim of this project is to establish whether bone acquisition in teenagers who have sustained a fracture and have low bone mass can be enhanced by changing environmental factors, such as mechanical loading. The effects of a twelve-month mechanical intervention on musculoskeletal development will be studied and the results will be compared to matched teenagers undergoing no intervention. This study also examines the possible relations between the cross-sectional properties of bone and circulating levels of IGF-I, IGF-binding protein-3, and IGF-I genotypes in teenagers ages 15 to 20 years with sport-related fractures. The possible relations between bone acquisition induced by mechanical stimulus and circulating levels of IGF-I and the IGF-I genotype will be assessed. The cross-sectional arm of this project was completed with 100 females in July, 2003. From this group, 48 subjects with low measurements for bone density or bone size were asked to enroll in phase two, the longitudinal arm. An intervention group and a control group have been formed; 24 females are using the vibration intervention system for 10 minutes/day and taking calcium 500 mg daily, 24 controls are taking calcium 500 mg daily.

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## INTRODUCTION

**This report, originally submitted in October, 2003, has been revised to incorporate the recommendations of the reviewers. Revisions have been added in bold typeface.**

Available data indicate that the genetic susceptibility for low bone mass is present very early in life. The aim of this project is to establish whether bone acquisition in teenagers who have sustained a fracture and have low bone mass can be enhanced by changing environmental factors, such as mechanical loading. The effects of a twelve-month mechanical intervention on musculoskeletal development in teenagers will be longitudinally studied and the results will be compared to matched groups of teenagers undergoing no intervention. The mechanical intervention will consist of brief exposure to low level (0.3g; 1g = earth gravitational field) high frequency (30-Hz) mechanical loading for 10 minutes every day. The cross-sectional properties of the bone make a substantial contribution to its strength. Data indicate that the cross-sectional dimensions of bone are important determinants of low-energy impact fractures in children, stress fractures in military recruits, and osteoporotic fractures in elderly women. Insulin-like growth factor-I (IGF-I), a major regulator of longitudinal bone growth, has also recently been shown to be an important determinant of cross-sectional bone growth. This study will examine the possible relations between the cross-sectional properties of bone and circulating levels of IGF-I, IGF-binding protein-3, and IGF-I genotypes in teenagers with fractures. The possible relations between bone acquisition induced by mechanical stimulus and circulating levels of IGF-I and the IGF-I genotype will also be assessed.

## BODY

Following approval by the local Internal Review Board (The Committee on Clinical Investigations) to conduct this study in June 2002, we commenced recruitment efforts to enroll teenage females who had suffered a low impact fracture and were treated in the Orthopaedics Department at Childrens Hospital Los Angeles (CHLA). While good response to recruiting efforts was received, the number of female patients between 16 to 18 with fractures was minimal. Recruitment modalities and the age range of participants were, therefore, expanded without changing the design or outcomes of the study. Teenagers between the ages of 15 to 20 years old who had sustained a low impact fracture were recruited through flier distribution from family members and friends of employees at CHLA, siblings and friends of patients at CHLA and local schools. Subsequently, a total of 100 female subjects were successfully enrolled and completed the cross-sectional phase of the study. Each participant underwent a physical examination to confirm completion of sexual development, anthropometric measurements, an x-ray of the left hand/wrist for skeletal age, blood draw for IGF-I, IGFBP-3 and genotyping, and measurements of bone and body composition obtained via computed tomography (CT) and dual energy x-ray absorptiometry (DXA). In addition, questionnaires were administered pertaining to dietary intake and physical activity of the participants. The cross-sectional arm in **100** female subjects was completed in July 2003.

**Baseline characteristics for most of the parameters studied are included in Table 1.** Calcium and sodium intakes on 100 female subjects have been analyzed and preliminary results indicate no correlations with bone density or body composition measurements either by DXA or CT. Serum samples have been transferred to the Maine Center for Osteoporosis Research for analysis and DNA has been extracted as per protocol. **We anticipate that hormone levels and DNA genotyping will be analyzed in these subjects by February 2004.**

**Table 1**
**Baseline Characteristics in 100 Female Subjects**

| Characteristics | Baseline Values* n = 100 |
|---|---|
| Age (yr) | 17.3 ± 1.38 |
| Weight (kg) | 65.5 ± 16.99 |
| Height (cm) | 162.3 ± 6.13 |
| Body Mass Index (kg/m$^2$) | 24.9 ± 6.19 |
| Skeletal Age (yr) | 17.2 ± 0.98 |
| CT - Vertebrae | |
| Cancellous Bone Density (mg/cm$^3$) | 177 ± 6.75 |
| Cross-sectional Area (cm$^2$) | 8.70 ± 1.19 |
| Vertebral Height (cm) | 2.36 ± 0.15 |
| Vertebral Volume (cm$^3$) | 20.59 ± 3.63 |
| CT - Femur | |
| Cortical Bone Density (mg/cm$^3$) | 1255 ± 37 |
| Cross-sectional Area (cm$^2$) | 5.08 ± 0.70 |
| Cortical Bone Area (cm$^2$) | 4.12 ± 0.52 |
| DXA - Lumbar Spine | |
| Bone Mineral Content (g) | 53.9 ± 8.52 |
| Bone Mineral Density (g/cm$^2$) | 0.99 ± 0.10 |
| DXA - Hip | |
| Bone Mineral Content (g) | 38.5 ± 6.97 |
| Bone Mineral Density (g/cm$^2$) | 1.05 ± 0.14 |
| DXA - Total Body | |
| Bone Mineral Content (g) | 2040 ± 298 |
| Bone Mineral Density (g/cm$^2$) | 1.10 ± 0.08 |
| Calcium Intake (mg/day) | 1227 ± 857 |
| Sodium Intake (mg/day) | 2870 ± 1823 |
| Physical Activity (hrs/wk; inc. walking) | 18.6 ± 12.24 |

**Values are mean ± SD*

**Subjects were stratified into quartiles of 25 subjects each based on values for bone mass. Those in the lowest quartiles were invited to participate in the longitudinal arm of this study and were randomized to either the mechanical intervention group or the control group.** The mechanical intervention phase in female subjects with low values for bone density or bone size commenced in August 2003. To date, 24 subjects are participating in the mechanical intervention group and 24 females are controls. On the day of enrollment into the longitudinal study and following the informed consent process, subjects were provided with a six months daily dosage supply of TUMS 500 mg and height, weight and trunk height were obtained. After six months, participants will be contacted via telephone for assessment of their dietary intake and physical activity via questionnaires. All participants in both the mechanical intervention group and in the control group are contacted via telephone on a weekly basis to ensure compliance with the study procedures. Subjects in the intervention group stand on the vibration system platform once a day for 10 minutes. Non-use (i.e., during vacations away from home, illness, etc.) is documented during weekly telephone contacts. In addition, the vibration system is equipped with a monitoring device to check usage, and monthly site inspections by a technician are performed to calibrate the equipment and download usage data. Technical problems with the equipment have been limited to minor issues regarding electrical outlets; no complaints have yet been registered by the users. Subjects in both intervention and control groups are asked to take a daily dose of TUMS 500 mg.

Due to unforeseen logistic constraints in recruitment and time, only the two main arms of this study, the mechanical intervention group and the control group, have started. Additionally, rather than risk loss of compliance by asking the participants to visit CHLA three times per week to use the mechanical stimulation device, the equipment was transported and installed in the homes of the 24 subjects in the intervention group. In this manner, participant's tasks are eased and their willingness to comply is enhanced for the considerable long term demands of this study. **Compliance with calcium intake was assessed by weekly telephone calls to subjects in the control and study groups, and was shown to be very high (> 90%). Compliance with the mechanical intervention was assessed subjectively in the same weekly telephone calls and objectively by monthly determinations of usage as indicated from an internal monitoring device installed in each platform. Table 2 shows compliance with mechanical intervention for four months, as requested by the reviewer. Overall, subjects used the mechanical intervention ~50% of the time (149.8 ±100.1 minutes/month). However, there was a great range in values; four subjects used the device < 10%, whereas another four used it 100%. It should be stressed that differences in compliance will be a variable included in the analysis of data. By the fourth month, no subjects had dropped out of the study.**

**The most important determinants of bone acquisition are events during puberty, and bone mass more than doubles during this brief period, reaching its peak soon after sexual maturity. Although we broadened the range of ages of subjects eligible for this study to include females between 15 to 20 years of age, at baseline all candidates underwent a physical examination to determine Tanner stage of development and all enrollees had**

**achieved sexual maturity as indicated by Tanner stage 5. The mean age of the female subjects enrolled in the longitudinal study was 18.7 at baseline and will be two years older at completion. Hence, the maturity range of the subjects will not affect the results of the project.**

**Recruitment of females for participation in this study was difficult and took longer than anticipated. Thus, to avoid additional delays, we commenced the longitudinal phase with only two arms. However, our intention is to proceed with the resistance intervention arm and we are recruiting an additional 44 female subjects to complete the n of 144 as indicated in the SOW.**

**Table 2**
**Mechanical Intervention Compliance in 24 Subjects**

| Subject ID# | Minutes of Use/Month* |
|---|---|
| A002 | 262 |
| A004 | 51 |
| A007 | 301 |
| A009 | 142 |
| A020 | 24 |
| A025 | 305 |
| A028 | 170 |
| A029 | 168 |
| A031 | 208 |
| A032 | 15 |
| A044 | 78 |
| A049 | 235 |
| A056 | 33 |
| A058 | 205 |
| A061 | 72 |
| A066 | 23 |
| A067 | 239 |
| A080 | 126 |
| A081 | 305 |
| A084 | 115 |
| A090 | 90 |
| A091 | 13 |
| A092 | 112 |
| A097 | 147 |
| A100 | 305 |

****Values reflect the mean over a 4-month period***

In October 2003, recruitment efforts for male subjects for the cross-sectional phase will begin. Given that we enrolled and completed the cross-sectional examinations in 100 females in a 12-month period, and that there is a much higher number of males 15 to 20 years old who have fractured, we anticipate successful recruitment of 144 male subjects and completion of the cross-sectional phase by August 2004. This time frame not only permits incorporation of the physical activity arm into the project, but it also coincides with the completion of the 12-month mechanical intervention arm in the females, which will allow us to transfer the vibration systems to the homes of male participants in this intervention group.

## KEY RESEARCH ACCOMPLISHMENTS

- HSRRB and IRB approvals.
- Successful completion of the cross-sectional phase in 100 female subjects.
- Successful implementation of the two-year longitudinal phase consisting of a mechanical intervention group and a control group in female subjects.
- Infrastructure in place to begin recruitment of male subjects for the cross-sectional phase.

## REPORTABLE OUTCOMES

- Preliminary analysis of the dietary intake showed no correlations between calcium or sodium intake assessed by dietary questionnaires with DXA or CT bone and body composition measurements in young females.

- Females 15 to 20 years of age who have sustained a low-impact fracture tend to be overweight when compared with females of the same age who have never fractured. Like previous studies, we found that girls with fractures are, for the most part, overweight [1,2]. This finding is especially provocative when coupled with the knowledge that, in the elderly, obesity is a known protector against osteoporotic fractures.

## CONCLUSIONS

None

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## APPENDICES

None